REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.21, attached as an Appendix is a version of the above amendments with markings to show changes made to the present application.

Creating focal genetic modifications in an intact animal is a powerful approach for studying the cellular interactions that underlie the development and function of tissues and organs. This method has been used to great advantage in *Drosophila*, facilitating the study of developmental questions relating to the autonomy of gene actions, restriction of cell fate and growth pattern of specific tissues. The approach involves the generation of genetic mosaics, tissues in which some cells differ from their neighbors by a single mutation, effecting either a gain or loss of function phenotype. Through the analysis of mutant and wild-type cells within mosaics patches, it is possible to draw inferences about interacting cells and in some cases the molecules and pathways subserving cellular communication.

The application of mosaic analysis to the study of nervous system function has the potential to yield a wealth of information because it should allow for an assessment of the function of particular gene products within individual cells that are part of a network. Thus, in tissues such as the nervous system where functional information resides not only in the nature and number of its constituent cells, but also in the manner in which they connect and temporally interact, it is essential that strategies be employed that neither unintentionally change the network nor eliminate some of the cellular constituents. Overall, the ideal strategy should permit stable genetic modification with precise temporal and spatial control.

Implicit in the use of genetic mosaic analysis is the ability to distinguish mutant from normal cells by the use of markers. The preferred marker is one which is gratuitous thus causing no cell damage, cell-autonomous so that cellular level resolution of mosaicism can be reliably scored, and having a short half-life to improve temporal analysis of the tissue after genetic modification.

Genetic mosaics have been generated in *Drosophila* by induction of mitotic recombination. Typically, radiation is used to induce DNA damage. In cells that have just completed DNA synthesis but have not yet divided, repair of the damage leads infrequently to homologous chromosome exchange. If the homologous chromosomes are appropriately marked, the resultant recombination event can be scored in daughter cells. More recently, high-frequency homologous chromosome exchange has been achieved by the use of the yeast

FLP/FRT system. The FLP gene product is a site specific recombinase that catalyzes recombination at FRT target DNA elements. Expression in flies of FLP induces recombination between FRT elements on homologous chromosomes. Inducible control of FLP by a heat shock promoter has been used in flies to grade the extent or recombination, thus modulating the extent of genetic mosaicism.

Genetic mosaics in flies have also been generated by inducing intramolecular chromosomal recombination with the FLP/FRT system. In one example of this strategy, a transgene is constructed such that it is inactivated by the insertion of a DNA sequence encoding a stop codon flanked by FRT sites. After induction of FLP the inactivating DNA cassette is 'flipped-out' allowing for the transcription of an mRNA that yields a translatable gene product. This approach could be used to generate gains or loss of function at any transcriptionally active transgene integration site or at a specific gene location targeted by homologous recombination.

Although the use of the FLP/FRT system in mammalian cells has been reported, a different recombination system, cre/*loxP*, has received wider attention and apparently greater success. The cre recombinase is bacteriophage P1-derived, and it interacts with its target site, *loxP*, a 34 bp element to produce site specific recombination. Using a binary approach in transgenic animals, investigators constructed and introduced separately into the germline of mice two different transgenes; the first, a strong promoter driving cre, and the second a recombinatorial substrate which contained two *loxP* sites. Crossing the two transgenic lines gave rise to compound heterozygotes in which recombination occurred in a highly efficient manner. The cre/*loxP* system has also been applied in embryonic stem (ES) cells to create deleted alleles by targeting a homologous locus with a construct that contains *loxP* elements flanking the region to be excised. With this approach the transient expression of cre produced a significant frequency of recombination events. Overall, the use of recombination systems in mice appears to satisfy the need for the efficient creation of stable genetic mosaics. However, the approach is significantly limited by constraints imposed by the characteristics of the promoter chosen to express the recombinase. Since every cell that expresses the recombinase will likely suffer a recombination event, it is difficult to use the system to generate mosaic tissues.

A need exists for a system to produce stable genetic mosaics where precise temporal and spatial control of gene expression can be obtained.

The rejection of claims 67-76 under 35 U.S.C. § 112 (1st para.) for failure to satisfy the written description requirement is respectfully traversed in view of the above amendments to the claims, replacing the word "human" with "mammal".

The rejection of claims 67-76 under 35 U.S.C. § 112 (1st para.), because the specification does not provide an enabling disclosure of how the present invention would be practiced to produce transgenic humans, is respectfully traversed.

As amended, the claims are now directed to transgenic mammals. Applicants submit that the production of transgenic mammals is fully enabled by the disclosure of the present application.

To the extent this rejection is maintained because the "transgenic mammal" limitation encompasses "transgenic humans", applicant respectfully disagrees with the U.S. Patent and Trademark Office's ("PTO") position. In the outstanding office action, the PTO states that elements of a gene construct to be transferred to a particular species of mammal are critical to successful expression of the transgene and must be designed on a case by case basis. The PTO notes that this is necessary, because expression of a transgene is effected by the site of integration and the random nature of transgene insertion within a mammal's genome. This entire line of argument is based on the asserted unpredictability of transgene expression when the transgene is microinjected into a mammal. However, this position is flawed, because microinjection is not the only technique used to produce transgenic mammals in accordance with the present invention.

It is well known that transgenic mammals can also be produced by transfection of embryonic stem cells instead of by pronuclear microinjection. Lamb, et. al., "YAC Transgenics and the Study of Genetics and Human Disease," Current Opin. Genet. & Devel. 5: 342-48 (1995)("Lamb")(attached hereto as Exhibit A); Kong, et. al., "Preparing Transgenic Animals with a Simplified Method of Morula Aggregation Using ES Cells," Lab Animal 29(3): 25-30 (2000)("Kong")(attached hereto as Exhibit B); and Wheeler, et. al., "Transgenic Technology and Applications in Swine," Theriogenol. 56: 1345-69 (2001)("Wheeler")(attached hereto as Exhibit C). The production of the transgenic mammals of the present invention by transfection of embryonal stem cells (instead of by pronuclear microinjection) is explicitly disclosed in the present application at page 16, lines 5-7.

In discussing pronuclear microinjection, Lamb, Kong, and Wheeler each note that one disadvantage of this procedure is the difficulty of making genetic insertions at precise locations. However, these references then proceed to reflect the well established view

that this problem can be overcome when transgenic mammals are produced by transfection of embryonic stem cells. In this regard, Lamb states:

> ES cells are totipotent embryonic cells that can be cultured indefinitely *in vitro* and can be genetically modified with precision, including the DNA sequence replacements and deletions utilized in gene 'knockouts' and even more subtle single base pair changes. Modified ES cells are used subsequently to produce chimeric mice. Transmission of ES cell DNA through the germline results in the production of transgenic mice containing the genetic modification.

Id. at page 342 (citations omitted). Kong states:

> Pleuripotent embryonic stem (ES) cells, commonly used to create gene knockout mice via homologous recombination, may be a convenient alternative technique for transgenic animal preparation. Preparing transgenic animals via ES cells offers several advantages. For example, the cells can be pre-selected for site of integration, low gene copy number, and high expression.

Id. at page 25 (citations omitted). See also Wheeler at page 1346. Thus, those skilled in the art fully recognize that the concerns noted by the PTO in support of its enablement rejection are not relevant when transfection of embryonic stem cells is used to produce transgenic animals. This technique is explicitly disclosed by the present application.

At page 1351, Wheeler notes that embryonic stem cells are available for a wide variety of mammalian species, including humans. Using such cells together with the constructs and techniques described in the present application and the knowledge possessed by those skilled in the art on how to produce transgenic mammals from embryonal stem cells, one of ordinary skill in the art would be fully able to carry out the present invention with all types of mammals, including humans. Tang, et. al., "A Cre/*lox*P-Deleter Transgenic Line in Mouse Strain 129S1/SvlmJ," Genesis 32: 199-200 (2002)(attached hereto as Exhibit D), Guo, et. al., "A Cre Recombinase Transgene with Mosaic, Widespread Tamoxifen-Inducible Action," Genesis 32: 8-18 (2002)(attached hereto at Exhibit E), Kaartinen, et. al., "Removal of the Floxed Neo Gene from a Conditional Knockout Allele by the Adenoviral Cre Recombinase In Vivo," Genesis 31: 126-29 (2001)(attached hereto at Exhibit F); and Gertsenstein, et. al., "ES Cell-Mediated Conditional Transgenesis," Methods in Molecular Biology 185: 285-307 (2002)(attached hereto as Exhibit G) all show that those skilled in the art have been able to successfully produce transgenic mammals,

from embryonic stem cells, with recombinatorial substrates like those claimed by applicant.

It is thus apparent that the present application would fully enable one of ordinary skill in the art to produce transgenic mammals, including transgenic humans, in accordance with the present invention. Accordingly, the non-enablement rejection under 35 U.S.C. § 112 (1st para.) cannot be maintained and, therefore, should be withdrawn.

Finally, it is noted that the present application was filed with an Information Disclosure Statement under 37 CFR §§ 1.976-1.98, listing 17 references on the accompanying PTO-1448 form. However, an initialed copy of that form was not returned with the outstanding office action. Applicant respectfully requests that the cited references be considered, that the PTO-1449 form (copy enclosed) be initialed to reflect such consideration, and that that initialed form be mailed to applicant with the next written communication from the PTO.

In view of all the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: April 4, 2003

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April 4, 2003 Date	Ruth R. Smith

In reference to the amendments made to the specification, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In the Claims:

Please amend claims 67-68, 71, and 73-74 as follows:

67. (Amended) A method of activating a gene to be expressed in a recombinatorial substrate, comprising:

providing a transgenic [human] mammal carrying a DNA molecule comprising a recombinatorial substrate, said recombinatorial substrate comprising:

a promoter element capable of promoting transcription of genes in the recombinatorial substrate;

a gene whose expression is to be controlled, said gene being positioned 3' to the promoter element to facilitate its transcription;

a terminator positioned 3' to said promoter and 5' to said gene whose expression is to be controlled to prevent transcription of genes 3' to said terminator; and

a first recombination site located 3' to said terminator and a second recombination site located 5' to said terminator, whereby treatment of said DNA molecule with a recombinase specific to the recombination sites removes said terminator from said DNA molecule, thereby activating the recombinatorial substrate and permitting transcription of said gene whose expression is to be controlled, wherein the transgenic [human] mammal has no gene encoding a recombinase,

introducing into the transgenic [human] mammal, through its somatic cells, a gene encoding a recombinase and

expressing said recombinase, which when expressed in the somatic cells, will promote the excision of DNA from said first recombination site to said second recombination site within the recombinatorial substrate and wherein activation of said gene whose expression is to be controlled confers a detectable and/or functional phenotype on the [human] mammal when expressed in the somatic cells of the [human] mammal.

68. (Amended) The method of claim 67, wherein said introducing comprises:

providing a vector comprising the gene encoding a recombinase and

introducing the vector directly into the somatic cells of the transgenic [human] mammal.

71. (Amended) The method of claim 67, wherein said introducing is carried out by delivering a nucleic acid molecule comprising the gene encoding a recombinase into the somatic cells of the transgenic [human] mammal by use of virosomes, liposomes, naked DNA, or particle bombardment.

73. (Amended) A method of activating a recombinatorial substrate, comprising:

providing a transgenic [human] mammal carrying a DNA molecule comprising a recombinatorial substrate, said recombinatorial substrate comprising:

a promoter element capable of promoting transcription of genes in the recombinatorial substrate,

a gene whose expression is to be controlled, said gene being positioned 3' to the promoter element to facilitate its transcription, and

a first recombination site located 3' to the gene whose expression is to be controlled and a second recombination site located 5' to the gene whose expression is to be controlled, whereby treatment of said DNA molecule with a recombinase specific to the recombination sites removes said gene whose expression is to be controlled from said DNA molecule, thereby activating the recombinatorial substrate and resulting in a loss of function of said gene whose expression is to be controlled, wherein the transgenic [human] mammal has no gene encoding a recombinase;

introducing into the transgenic [human] mammal, through its somatic cells, a gene encoding a recombinase, and

expressing said recombinase, which when expressed in the somatic cells, will promote the excision of DNA from said first recombination site to said second recombination

site within the recombinatorial substrate and wherein activation of said recombinatorial substrate confers a detectable and/or functional phenotype on the [human] mammal.

74. (Amended) The method of claim 73, wherein said introducing comprises:

providing a vector comprising the gene encoding a recombinase; and

introducing the vector directly into the somatic cells of the transgenic [human] mammal.